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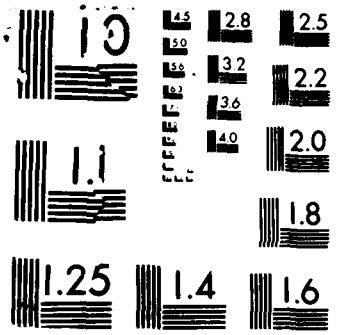
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STUDY OF AFRICAN TRYPANOSOMIASIS

Annual Report
(1 October 1979-30 September 1980)

I. Muriithi

September 30, 1980

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The pathogenesis of Trypanosoma brucei rhodesiense was studied in 42 cattle experimentally infected with different isolates from Kenya, Ethiopia and Tanzania. Nineteen of the cattle died or were terminated in extremis 3-60 months post inoculation. Nine of 11 isolates tested produced central nervous system (CNS) disorder in the cattle. The CNS disorders developed early (3 months) in some animals and later (60 months) in others. Parasites were difficult to find on wet blood smears or by subinoculations of blood

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after the fourther month of infection. Parasites persisted in lymph nodes, however. It appears that the bovine is a suitable model for T. b. rhodesiense infection with syndromes similar to those in human infections.

Berenil(R) treatment of hosts and subsequent transmission of T. congolense and T. vivax by tsetse flies was investigated. No significant differences in transmission were noted between flies fed on treated animals and those fed on untreated animals.

The complement fixation (CF) test and the micro-ELISA were compared in the diagnosis of visceral leishmaniasis. The CF was highly specific, reproducible and apparently very sensitive, both in field and laboratory tests. Antibody titers were detected up to 5 months after cure. The micro-ELISA failed to differentiate among various types of antisera and does not appear to be useful in kala-azar diagnosis.

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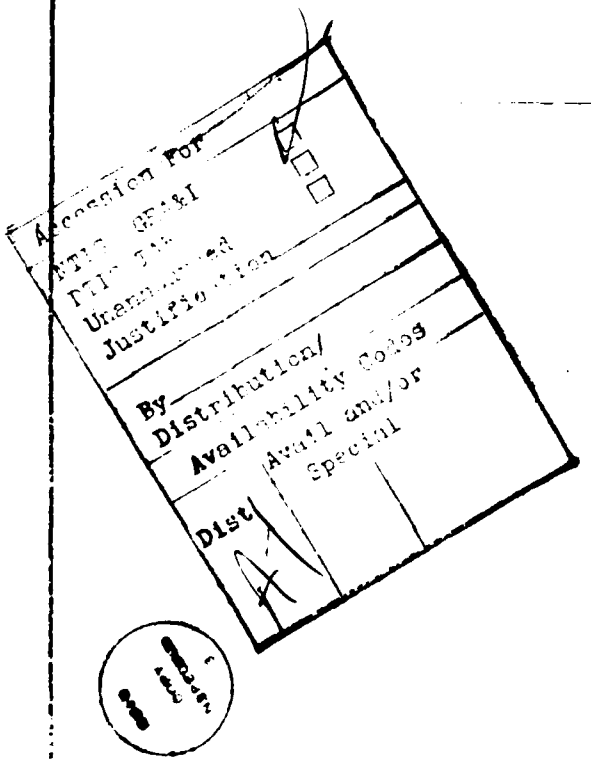

Trypanosoma brucei rhodesiense: Experimental Infections in Cattle

INTRODUCTION

Organisms of the Trypanosoma brucei subgroup have generally been regarded as mildly pathogenic for cattle (Stephens, 1970). In the instance of T. b. rhodesiense specifically, experimental infections of from 4 to 9 months have been reported in cattle but without obvious pathogenicity (Wild and French, 1945). Carmichael (1933) however described the death of 9 to 12 animals experimentally infected with T. rhodesiense. Cattle have been shown to naturally harbor this parasite and have been incriminated as a reservoir host (Onyango, 1968). The conflicting nature of the reported pathogenesis in experimental animals and the uncertainty of what occurs under natural conditions led us to investigate laboratory induced infections of T. b. rhodesiense in the bovine in an attempt to clarify the pathogenesis of the parasite for this host.

MATERIALS AND METHODS

Experimental animals

Cattle used in these studies were selected from the Veterinary Research Laboratory farm at Kabete, Kenya. Random breed albino rats and mice were used for subinoculation and for maintaining the trypanosome strains.

Parasites

Eleven isolates of T. b. rhodesiense were employed. Eight were obtained from patients at the Homa Bay District Hospital, South Nyanza, Kenya (LVH), one from Samia, Kenya (SM), one was isolated in Gambela, Ethiopia (ETH), while the other, (Wellcome C-T), was isolated in Tanzania in 1934 and has been maintained in laboratory rodents since then.

Trypanosomes were collected from infected rats by cardiac puncture, counted in a hemocytometer and subsequently diluted with phosphate buffered saline (containing 5% glucose, 10% fetal calf serum) to 1×10^4 per ml. Animals were all injected via the jugular vein with 1 ml. of inoculum.

Collection of specimens

Blood for smears was obtained by puncturing the tip of the tail while blood for hematological determinations was collected from the jugular vein. Lymph node aspirates were usually obtained from a prescapular lymph node and cerebrospinal fluid was collected aseptically through a spinal needle inserted at the lumbar sacral junction.

Laboratory procedures

Routine hematological methods (Wellde et al. 1974) were employed. Total serum proteins were determined by the Biuret method and serum electrophoresis on cellulose acetate was done using the Beckman Microzone technique. Immunoglobulin levels were estimated using commercially prepared Mancini plates (Miles Laboratories, Elkhart, Indiana). Glucose levels determined as previously described by Williams et. al (1966). Complement fixation tests were done following the method of Staak and Lohding (1979). In an attempt to identify other agents in cerebrospinal fluid (CSF) aliquots of CSF were inoculated into embryonic bovine kidney cell cultures, blood agar and brain heart infusion broth.

RESULTS

Forty two animals were infected with trypanosome isolates that originated in Kenya, Ethiopia or Tanzania and to date, nineteen animals have died or were terminated *in extremis* 3 to 60 months post inoculation (Table 1). The clinical syndrome was characterized by fever, weight loss, leucopenia followed by leucocytosis, hypertrophy of palpable lymph nodes and by signs of a central nervous system disturbance (Table 2). Trypanosomes were found in the blood during the first 8 months of infection, but subsequently they could only be isolated from lymph nodes or cerebrospinal fluid (Table 3). Generally a mild to moderate anemia developed as the infection progressed (Table 4). Total serum proteins increased during the early months of infection but subsided in some animals prior to death. Increased levels of protein were caused by elevated gamma globulin levels. Albumin levels declined during the course of infection while no significant changes were noted in the alpha and beta globulin fractions. Analysis of serum immunoglobulin levels indicated increases in both IgG and IgM. Cerebrospinal fluid from animals experiencing central nervous system disturbances had a pleocytosis. Total protein levels in the CSF were also increased and were due to a rise in immunoglobulin G primarily. Complement fixing antibodies were also detected in the CSF of infected animals (Table 5).

Gross observations included thickened dull grey meninges over the dorsal aspects of the brain and prominent lymph and hemolymphadenopathy. Histologically, a severe meningoencephalitis was the salient histological feature. The results from 10 animals are summarized in Table 6. The most severe changes were noted in the white matter of the central nervous system, where mixed perivascular

plasmacytic and lymphocytic infiltrates and focal and diffuse gliosis were important features. Demyelination was almost exclusively limited to perivascular areas. Although involvement of the spinal and peripheral nerves was minimal, limited infiltrates of inflammatory cells in the neural sheath and perivascular spaces were found on thorough search. In three animals autopsied between 84 and 108 days post inoculation a moderate to severe pancarditis was found. Myocytolysis and sarcolemmal cell hyperplasia accompanied by infiltrates of macrophages, lymphocytes and plasma cells were evident. The epicardium and endocardium also had infiltrates of inflammatory cells. The lungs of these animals had large numbers of hemosiderin-laden macrophages in the alveolar walls.

DISCUSSION

With the exception of two isolates, T. b. rhodesiense produced a marked central nervous system disorder in a significant number of cattle. The reasons why some animals develop CNS disease are not apparent from this study; however, the process may be rapid (3 mos) or extended (60 mos). Detection of parasites in infected cattle was difficult after the fourth month of infection if blood smears or subinoculations of blood alone were used. Examination of Geimsa stained lymph node smears improved detection but subinoculation of lymph node aspirates to rodents was most effective. Parasites were shown to persist in lymph nodes for extended periods of time as occurs in T. b. gambiense infections in man. The presence of severe meningioencephalites with the most extensive lesions in the white matter of the central nervous system is compatable with the leucoencephalitis observed in man with trypanosomias, (Caldwell, 1937; Manuelidis, 1967). The myocardial lesions in animals dying

relatively early after infection are consistent with the cardiac syndrome associated with T. b rhodesiense in man (Ormerod, 1970). The present work demonstrates that the bovine is a useful model for T. b. rhodesiense infection. However, we are attempting to produce a more predictable model in the bovine by intrathecal inoculation of T. b rhodesiense and to determine whether or not the experimental disease is similar to the course of infection in the field.

Table 1

BOVINES INFECTED WITH TRYPANOSOMA [illegible] ISOLATES

Parasite Designation	Date and Place Isolated	Number Animals Infected	Number with central nervous system disorder
Wellcome-CT	1934-Tanzania	9	1
LVH-1	1972-Kenya	2	2
LVH-2	1973-Kenya	2	2
LVH-9	1974-Kenya	1	1
LVH-12	1974-Kenya	10	5
LVH-28	1976-Kenya	4[a]	1
LVH-29	1976-Kenya	4	3
LVH-30	1976-Kenya	4[b]	2
LVH-34	1976-Kenya	2	0
SM-1	1976-Kenya	2	0
ETH-1	1974-Ethiopia	2	1

a. 3 animals killed accidently

b. 1 animal died of bloat

Table 2

CATTLE DEVELOPING SEVERE CENTRAL NERVOUS SYSTEM DISEASE CAUSED BY TRYPANOSOMA BRUCEI RHODESIENSE

Animal No.	Isolate	Clinical[a] Signs	Duration (Days)	Trypanosomes by subinoculation at necropsy		
				Blood	Lymph Node	CSF
8901	LVH-29	1-5	85	+	+	+
8888	LVH-29	1-5	92	+	+	+
15	LVH-29	1-6	108	+	+	+
268	WELLCOME	1-5,9,10	178	-	N.D.	N.D.
6882	LVH-1	1-5	227	+	+	-b
7859	LVH-12	1-6,9	301	+	+	+
7307	LVH-9	1-5	336	-	+	+
243	LVH-1	1-5,7	582	-	N.D.	N.D.
12	LVH-29	1-6,8,10	585	-	-	+
8898	LVH-30	1-5,8,9	656	-	+	+
8601	LVH-12	1-7	704	-	+	-b
7304	LVH-2	1-7	715	-	+	-
8602	LVH-12	1-7	994	-	N.D.	N.D.
8606	LVH-12	1-8	1005	-	-	-c
313	LVH-12	1-6	1005	-	+	-b
8903	LVH-30	1-8	1125	-	+	+
7303	LVH-2	1-7	1410	-	+	-
7302	LVH-1	1-8	1613	-	+	+

[a] 1. Weight loss
2. Weakness
3. Akinesia
4. Ataxia
5. Salivation
6. Hypersensitivity
7. Tremor
8. Blindness
9. Circling
10. Opisthotonus

[b] Minced brain tissue +

[c] Minced brain tissue -

N.D - Not done

LOCATION OF TRYPANOSOMES AND EFFICACY OF VARIOUS DIAGNOSTIC METHODS IN TRYPANOSOMA RHODESIENSE INFECTED CATTLE

		Months post infection											
		0	1	4	5	6	7	8	12	16	20	24	28
Animals positive for trypanosomes by:	Blood Smear	0	10	0	0	0	0	0	0	0	0	0	0
	Blood Subinoculation	0	10	8	2	1	1	0	0	0	0	0	0
	Lymph Node Smear	0	N.D.	5	5	7	4	2	3	2	2	0	1
	Lymph Node Subinoculation	0	N.D.	10	10	10	9	7	5	4	3	1	1
	No. Animals Remaining	10	10	10	10	10	10	10	9	9	9	8	8

Table 4

Hematology and Weight Changes in Animals
Developing Central Nervous System Disorder

Animal No	Days Survival	Pre-Infection PCV[a]	Pre-Infection WBC[b]	Pre-Infection WT[c]	Terminal PCV[a]	Terminal WBC[b]	Terminal WT[c]
8901	85	30	8.7	418	18	15.5	308
8588	92	36	13.4	451	23	16.1	297
16	108	36	12.9	484	26	15.1	297
286	178	31	14.6	467	27	16.0	340
6882	227	29	11.2	420	20	9.4	325
7859	301	34	15.4	215	26	16.9	290
7307	336	33	13.5	360	19	15.5	462
243	582	28	9.6	325	26	18.7	370
12	585	32	13.6	517	26	13.8	495
8898	656	30	14.6	451	33	21.0	605
8801	704	32	12.9	290	26	30.1	495
7304	715	28	12.6	400	33	20.7	406
8602	994	30	12.4	260	28	6.6	451
8606	1005	31	16.8	290	28	9.8	528
313	1005	32	21.8	340	20	16.8	528
8903	1125	34	9.7	508	20	23.0	616
7303	1410	33	11.9	475	25	13.3	924
7302	1613	35	10.3	580	18	17.3	693
	Mean	31.9	13.1	403	24.7	16.3	472
	Range	28-36	8.7-21.8	215-580	18-33	6.6-30.1	297-924

a. Packed cell volume (%)

b. White blood cell count /mm^3 x 10^{-3}

c. Weight in grams

Table 3: Sensitivity and Specificity of the [illegible] with Human Sera known to be Antibody Positive

Diagnostic Status of Serum Donor	Number Tested	Number Reacting at Titer							
		1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
South American Cutaneous Leishmaniasis	8								
Visceral Leishmaniasis	122		8	25	32	22	26	9	
Syphilis	6								
Trypanosomiasis									
African	5								
American	5								
Malaria	21								
Echinococcosis	1								
Shistosomiasis	10								
Taeniasis	1								
Paragonimiasis	1								
Meningitis	9								
Onchocherciasis	5								
Tuberculosis	3								
Normal Control	19								
Clonorchiasis	1								
Typhoid	1								
Pneumonia	6								

Table 6. Reproducibility of the CF Test With Single Sera Run on Different Days With Different Aliquots of Antigen and Sheep Red Blood Cells

Serum Source	No. Times Tested	No. Times Given Titer Obtained						
		0	5	10	20	40	80	160
Tuberculosis	6	6						
American Trypanosomiasis	2	2						
African Trypanosomiasis	3	3						
Clonorchiasis	2	2						
Echinococcosis	2	2						
Taeniasis	2	2						
Paragonimiasis	2	2						
Typhoid	3	3						
Onchocerciasis	3	3						
Pneumonia	4	4						
Malaria	4	4						
Visceral leishmaniasis	6					3	2	1
Visceral leishmaniasis	17				3	7	7	
Visceral leishmaniasis	6					3	2	1
Normal Control	29	29						

Table 1. Example of Complement Fixation Test for Visceral Leishmaniasis. A "plus 4" Reaction is Considered Positive.

Antigen	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
1:4	4	4	4	4	4	3	3	2	
1:8	4	4	4	3	3	2	2	2	
1:16	4	4	3	2	Tr*	Tr	Tr	Tr	
1:32	4	3	2	Tr	Tr	Tr	Tr	Tr	
1:64	1	Tr	Tr	Tr	-	-	-	-	

* Trace reaction

STAGE 1:

1. Patient's serum added in various dilutions.
2. L. donovani antigen added in various dilutions.
3. Guinea pig complement added (activity titrated for each test).
4. Incubated at $4^{o}C$ overnight.

STAGE 2:

1. Added .05 ml of Sheep Red Blood Cells/Rabbit anti-SRBC (1 Vol Hemolysin (1:1500).
2. Sealed the plate.
3. Incubated at $37^{o}C$ for 30 min.
4. Removed and read after 1 hr.

However, if the CF test proves to be efficient in kala-azar diagnosis we can expect to see additional CF positive patients (presumable early cases) with no detectable parasites.

Some kala-azar patients had titers of 1:1280 by CFT at an antigen dilution of 1:4, while at an antigen dilution of 1:16, titers of 1:320 were not uncommon. Of 60 parasitilogically proven cases, all had detectable CF antibody titers. These titers were detectable upon admission, through the course of treatment and for up to five months after presumed cure and discharge.

In summary, the CF test appeared promising for use in epidemiologic and zoonotic investigations because: (1) it was sensitive in detecting all parasitologically confirmed cases; (2) it was specific, since sera from patients with a wide variety of different diseases did not cross-react; (3) titers were detectable up to 5 months after patient cure.

The micro-ELISA was used for diagnosis of leishmaniasis by Hommel et al.[4] by Sells and Burton[5]. Both[4,5] used promastigote antigen as we did but both used the alkaline phosphatase technique instead of the peroxidase 5-amino-salicyclic acid method we used. Hommel et al.[4] tested canine and human serum and stated that it was easier to differentiate positives from negatives among canine sera than among human sera. Sells and Burton[5] reported cross reactivity between Chagas disease sera and leprosy sera. Perhaps the failure of the micro-ELISA to differentiate among the various sera tested was due to an alteration of the configuration or binding sites of our antigen in adhering the antigen to the plastic. However, specificity was not improved by antigen purification with techniques ranging from various affinity column chromatographic procedures to the protein "salting out" procedure. The micro ELISA technique as tested does not appear to be useful in kala-azar diagnosis.

RESULTS

Complement Fixation Test

When three positive leishmania sera were run on 6, 17 and 6 different occasions respectively, they were always positive and within 2 dilutions of each other (Table 2). The reproducibility is equally true for patients with other diseases - that is no detectable titer was apparent when tested on multiple occasions. As shown in Table 3, there was no cross reactivity with sera that were antibody positive for other diseases, and all 122 visceral leishmaniasis serum samples were CFT positive, with titers ranging from 1:10 to 1:320.

Micro-ELISA

Figure 1 shows the optical density (OD) values obtained from the serum samples tested with the micro ELISA. The test did not differentiate among sera of leishmaniasis, malaria, shistosomiasis, American trypanosomiasis or syphilis patients.

DISCUSSION:

Our unpublished data collected in a field survey near Masinga, Kenya showed that of 190 school children, 189 children had no CF titers while one was positive. Two children appeared ill upon clinical examination, had splenomegaly, low hemoglobins and PCV's. One of these was the CF positive child. When brought into the hospital, the child with no CF titer was diagnosed as having malaria, treated and discharged. The other CF positive child, was carefully examined and though 3 bone marrows were performed, no parasites were detected. She was placed on pentostam and improved clinically. This was the only patient with a positive CFT and no detectable parasites.

MICRO-ELISA

The enzyme-linked immunosorbent assay (ELISA) test used was that described by Voller et al[4], except that we used peroxidase 5-aminosalicylic acid method instead of the alkaline phosphatase technique. Specificity was determined by comparing known kala-azar positive sera and 5 other types of antisera along with normal human serum.

preparation was adjusted to 20 times the packed cell volume and sonicated at 20 KHz for 2 minutes. The sonicated material was centrifuged in the cold at high speed for 60 minutes and the supernatant was recovered and adjusted to a protein concentration of 4-5 mg/ml.

Complement Fixation Test

The CF test was performed on microtiter plates as outlined in Table 1. The antigen was diluted from 1:4 to 1:64, and the test serum was diluted from 1:5 to 1:1280. Normal non-infected controls and anti-complementary activity was assayed at the same time. Serum from a kala-azar patient and then the antigen was added in various dilutions. Guinea pig complement was added, and the plates were incubated at $4^{o}C$ for 24 hrs. After incubation, 0.05 ml of a sheep red blood cell (SRBC)/rabbit anti-SRBC suspension was added to each well of the micro-titer plate. This suspension was prepared by mixing one volume of a 2% suspension of SRBC's with 1 volume of hemolysin diluted 1:1500. Each plate was sealed and incubated at $37^{o}C$ for 30 min and then held for 1 hr. at room temperature before being read. A "plus 4" represented no hemolysis and was considered a positive CF test (Table 1). To evaluate the reproducability of the CF test, different batches of antigen, complement, hemolysin and SRBC's were used in repeated tests. Sera from patients with a variety of bacterial, protozoal, helminthic diseases were tested simultaneously. The tests were run at a 1:16 dilution of antigen. Specificity was evaluated by running CF tests on human sera known to be positive for cutaneous leishmaniasis, American and African trypanosomiasis and various other diseases. Tests were run at a 1:16 antigen dilution on a total of 224 serum samples, 122 of which were from visceral leishmaniasis patients.

COMPARISON OF THE COMPLEMENT FIXATION AND MICRO-ELISA IN THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN KENYA

INTRODUCTION:

Diagnosis of visceral leishmaniasis often depends on demonstration of organisms in stained smears or culture material obtained from the bone marrow, liver or spleen. The advantage of these procedures is that they allow visual observation of parasites obtained from a patient. However, these methods can be time consuming and may require further biopsy or aspirate for additional material if the first smear or culture is negative. The complement fixation (CF) test was first used for the serologic diagnosis of kala-azar in 1926[1]. Sadun[2] considered the CF test of limited routine diagnostic use, while San Gupta[3] considered it the test of choice for early diagnosis of kala-azar. Tests such as hemagglutination, direct agglutination, immuno-electrophoresis, gel diffusion and fluorescent antibody have been criticized for lacking specificity or sensitivity or have not been adequately tested in kala-azar diagnosis. The micro-ELISA has been effective in the diagnosis of other diseases but has not been tested sufficiently in the diagnosis of ~~kala-azar~~. In the present study we compared the CF test with the micro-ELISA in the diagnosis of kala-azar.

MATERIALS AND METHODS:

Antigen Preparation

Antigen for both tests was prepared as follows: a strain of Leishmania donovani isolated from a patient in Kenya was grown in Schneider's culture media supplemented with 30% fetal bovine serum. Organisms were harvested by centrifugation and washed 3 times with normal physiologic saline. The

for those fed on a Berenil(R) treated animal and 98% for the control flies. Neither the T. congolense nor the T. vivax differences were statistically significant (P 0.05).

DISCUSSION

It does not appear that Berenil(R) treatment of host animals can affect the infection rate in the vector. The flies may not be taking up sufficient Berenil(R) to affect the parasite, or perhaps the metacyclic form is not susceptible to the drug. It is likely that at least some level of Berenil(R) reached the parasites in the hypopharyn of the fly. No further studies of this type are indicated at the present time.

Berenil(R) Treatment of Hosts and Transmission of Trypanosoma congolense and Trypanosoma vivax by Glossina morsitans.

In previous immunologic studies (Grant Report 1979) Glossina morsitans cyclically infected with Trypanosoma congolense were fed on Berenil(R) treated steers. These flies later failed to transmit T. congolense when fed on an untreated bovine. Studies were conducted to determine if (1) subsequent fly transmission is interrupted when infected flies are exposed to a treated host and (2) infected flies are cleared of metacyclics when fed on a treated host.

MATERIALS AND METHODS

Twelve cages of 20 G. morsitans each were fed from emergence on a steer infected with the Trans-Mara strain of T. congolense. When the flies were 26-30 days old, the cages were fed on a non-infected steer for 4 days to eliminate the possibility of mechanically acquired (non-cyclic) infection. Half of the flies in each cage were dissected to determine the infection rate prior to exposure to a treated host. The cages were fed on normal mice to demonstrate that the remaining flies were capable of transmission of T. congolense. For the next 10 feedings, 6 of the 12 cages were fed on steers treated with Berenil(R) at various rates. The 6 remaining cages served as controls and were fed on a normal steer for 10 feedings. These same proceedures were followed for flies exposed to Trypanosoma vivax infection.

RESULTS

The infection rate among the T. congolense exposed flies that were fed on a Berenil(R) treated animal was 11.7%, while that of the control flies; was 15%. Among the flies exposed to T. vivax, the infection rates were 94%

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TABLE 6

SUMMARY OF THE HISTOLOGICAL CHANGES IN THE CNS OF CATTLE INFECTED WITH TRYPANOSOMA RHODESIENSE

ANIMAL	CERVICAL SPINAL CD.	CEREBELLUM	MIDBRAIN	CORTEX-OCCIPITAL LB.	BASAL GANGLIA	MIDCORTEX	CORTEX-OLFACTORY	PITUITARY	SEE COMMEN
16	1,3,5	1,3,5	1,3,5,6	1,3-5,8	1,3-6,8	1,3,5,8,9	1,3,5,8	1,3,7	A
243	1,3,4,7	1,3-8	1,3-5,7,9	1-9,11	1-11	1-9,11,12	1-5,7,9	3,4,8,10	B
268	N/A	1,3-5,7,8	1,3,5,7	N/A	1-8,10,12	1-3,5,7,8	N/A	3,4,8,13	C
6882	3	1,3,5,7	3,7	1,3	3	N/A	N/A	3,8,13	D
7304	1,3,4	1,3-5,8,10	1,3-5,7	1-10	1-3,5-8, 10-12	1-5,7-9,11	1-9	3,4,8,13	E
7307	1,3	1,3,5,7,8	1,3,6,7,11	1,3-5,8	1,3	1-5,8,9,11	1,3-5,8	3,13	F
7859	1,3,5,7	1,3,5-8	1-8	1-10	1-11	1-3,5-10,12	1-10	1,3,7	G
8601	1,3-5,7,14	1,3-8	1,3-5,7,8, 14,15	1-5,7-9,15	1,3-9,15	1-11,15	1-9,15	1,3,4,8	H
8888	1	1,3	1,3,5	1,3-5	1,3,5	1,3,5	1,3-5	1,3,7	I
8901	N/A	1,3	1,3	N/A	N/L	N/A	1	1,3,7	J

N/A: Not available or tissue sections not identified
N/L: No lesions
1: Meningeal infiltrations
2: Subpial gliosis
3: Perivascular infiltrates
4: Mott's cells
5: Vasculitis
6: Periarterial edema
7: Gliosis, focal
8: Gliosis, diffuse
9: Gemistocytic astrocytes
10: Malacia or Gitter cells
11: Vacuolation and/or cyst formation
12: Subependymal gliosis and/or malacia
13: Colloid cysts adenohypophysis
14: Neuronal degeneration in areas of intense infiltr
15: Perivascular hemosiderin

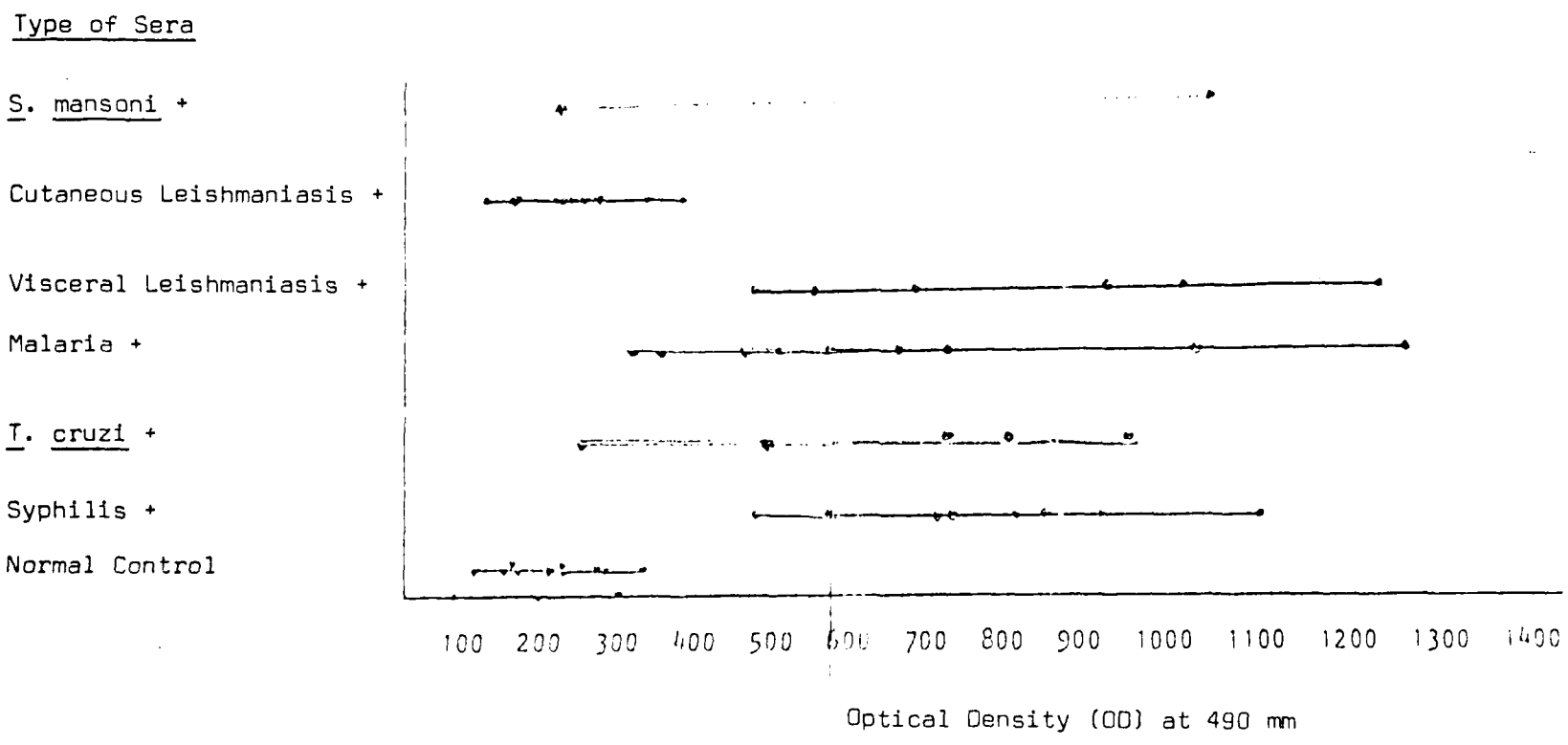
Table 5

CEREBROSPINAL FLUID COMPONENTS ($\pm$ 1SD)

Component	Infected (13)	Control (19)
Leucocytes/mm^3	400 ± 100	6 ± 3
Total Protein (mg/100ml)	136 ± 78	46 ± 9
IgG (mg/100ml)	142 ± 67	Not Detectable
IgM (mg/100ml)	10 ± 21	Not Detectable
CF Antibodies	Pos.	Neg.
Glucose (mg/100ml)	35 ± 9	45 ± 6
Bacteriology	Neg.	Neg.
Virology	Neg.	Neg.

Figure 1

Specificity Testing of Micro-ELISA in the Diagnosis of Visceral Leishmaniasis.



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